OCHROMONAS MONICIS SP. NOV., A PARTICLE FEEDER WITH BACTERIAL ENDOSYMBIONTS

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ABSTRACT. — *Ochromonas monicis* sp. nov. was isolated from a saline pool at the coast of the North Sea. The Single plastid with stigma and pyrenoid, and the simple strands ejected from the mucoid bodies, are the major characteristics. Both flagella are involved in the seizure of bacteria on which the species feeds.

Several galleries filled with cytoplasm penetrate the pyrenoid. Endosymbiontic bacteria were found inside the endoplasmic reticulum.

The author suggest that the endosymbiontic bacteria enable the species to survive in vitamin-depleted water, and that the species turns to feeding on bacteria when phosphorus or nitrogen in the water are depleted.

RÉSUMÉ. — *Ochromonas monicis* sp. nov. a été isolée à partir d'une mare salée de la côte de la Mer du Nord. Cette espèce est caractérisée par un seul plaste avec stigma et pyrénoide ainsi que par la présence de cordons muqueux simples rejetés par les corps muqueux. Les deux flagelles servent à capturer les bactéries dont se nourrit la cellule. Plusieurs galeries remplies de cytoplasme pénètrent le pyrénoide. Des bactéries endosymbiotiques sont présentes dans le réticulum endoplasmique.

Les auteurs pensent que ces bactéries endosymbiotiques permettent à la cellule de survivre dans des eaux pauvres en vitamines, et que l'algue se nourrit de bactéries lorsque les teneurs des eaux en phosphore ou en azote sont faibles.

INTRODUCTION

After the genus *Ochromonas* was established in 1887, over 80 species have been described in this taxon (RIETH, 1970) although, with few exceptions, the features of these flagellates are too variable for species recognition. KALINA (1964) united 8 species into one, *O. crenata* Klebs, a fact suggesting that a critical study of cultured material from the whole genus would indeed bring more

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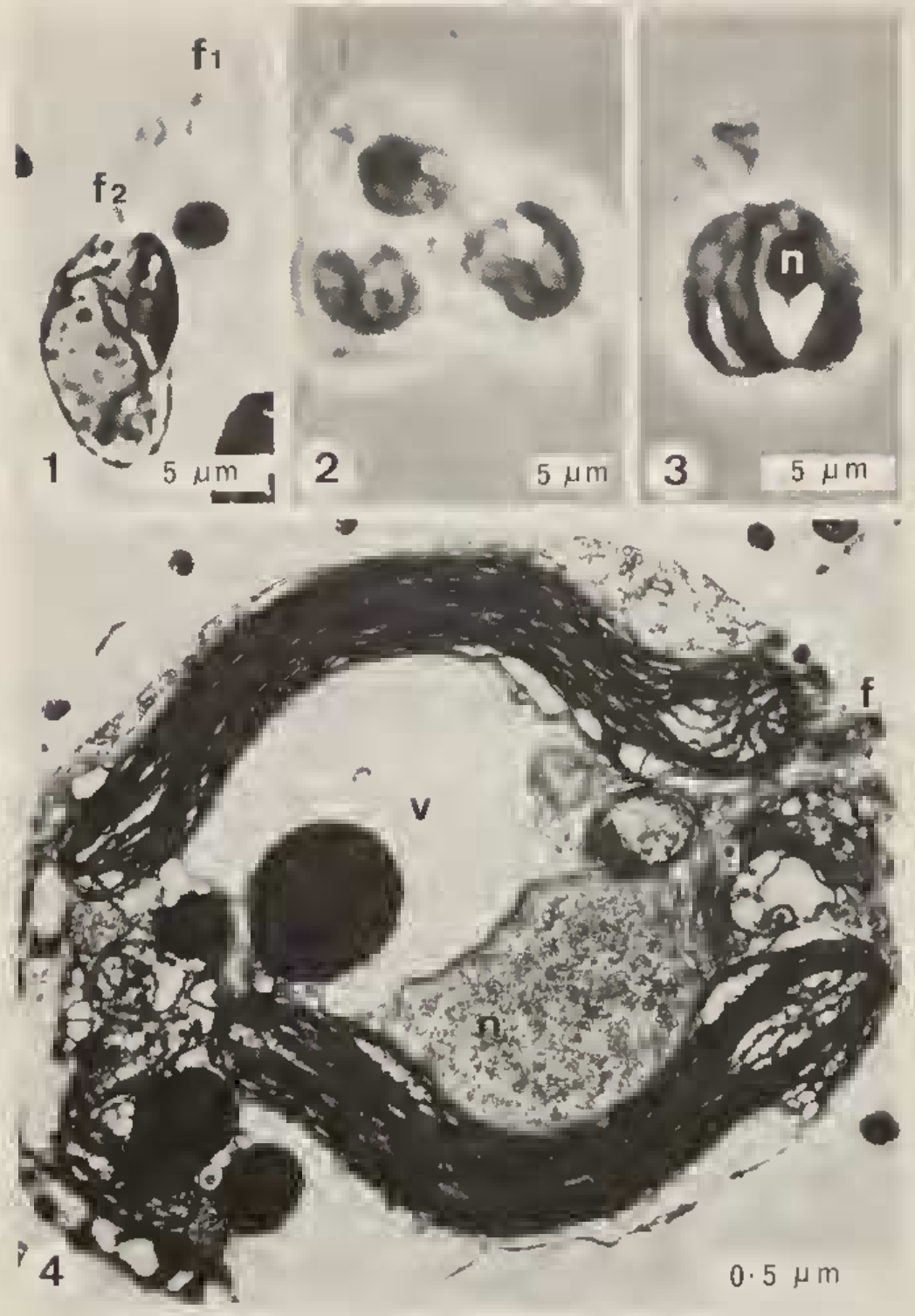

Plate I : light micrographs 1-3, electron micrograph 4. — Fig. 1 : Living cell, strechted to swim away. Long flagellum (f1), short flagellum (f2), pyrenoid (arrow) and several mucoid bodies. Clear field. — Fig. 2 : Three cells, fixed and stained with gentian violet. Several ejected strands of mucus visible in lower half of micrograph. Phase contrast. — Fig. 3 : Single cell, fixed and stained with Giemsa. Long flagellum artificially convoluted; stigma at the base of short flagellum; nucleus (n) covering pyrenoid; some bulging vesicles at the circumference of the cell. Phase contrast. — Fig. 4 : Thin section, circa longitudinal, showing two profiles of the plastid, nucleus (n), Golgi (g), flagellum (f) cut at the point of insertion, chrysolaminarin vacuole (v).

species into synonymy. Pending such a study, it seems unattractive to add one more species to the genus *Ochromonas*. For all that, we introduce the new species, *Ochromonas monicis*, because the presence of a pyrenoid in the material examined by us is a clear-cut feature that is uncommon in this genus. The new species is marine. *Ochromonas* is primarily a fresh water genus. RIETH (1970) mentioned 7 or 8 species only from saline habitats, and PARKE & GREEN (1976) 6 species from the seas around the British Islands. The presence of endosymbiontic bacteria in this bacteria-eating species is another reason for writing this paper. Recently the bearing of endosymbiosis and phagocytosis in protozoa on the theory of the food web has received the attention of marine ecologists. The kinds of endosymbiosis and phagocytosis found in *Ochromonas monicis* are probably equally wide-spread as those found in protozoa, but the effetcs on the flow of nutrients will be different.

MATERIAL AND METHODS

A strain of *Ochromonas* was isolated from a littoral pool on the Dutch Wadden island Schiermonnikoog. This pool, which has disappeared later on, received seawater during the winter storms, and received fresh water draining from the nearby dunes throughout the year. It consequently had a fluctuating salinity. The strain was cultivated in a seawater medium enriched with leaf-mould extract, at 17°C and 16 hours diurnal light period, provided by fluorescent tubes.

Living and fixed material was examined with the light microscope. Fixation for light microscopy was done with a mixture of acrolein, glutaraldehyde and tannic acid, according to a method described by Van der VEER (1982). Part of this material was dried on slides and stained with gentian violet, according to methods described in the same paper, before being mounted in DePex. Another part of the fixed material was – after several rinses with demineralized water to remove the fixative – suspended in Giemsa stain, in which it was left 5 minutes. After that, it was dehydrated in ethanol, and rinsed several times in 99 % ethanol to remove excess stain, before being transferred to euparal and mounted on slides.

For electron microscopy, material was fixed for 30 minutes in a cold osmium tetroxide solution containing 0.25 M sucrose and buffered by 0.1 M sodium cacodylate to pH 6.9. The cells were dehydrated in ethanol, followed by acetone and embedded in Vestopal. Sections were successively stained with lead citrate and uranyl acetate and examined with a Phillips EM 300.

LIGHT MICROSCOPY

Free swimming individuals are oblong conical, or nearly ellipsoid with a dorsal flattening (Pl. I, fig. 1). Some cells get quite irregular shapes under pressure or the coverslip. Attached cells are rounded, and when feeding on particulate matter, are distended by the presence of digestive vacuoles.

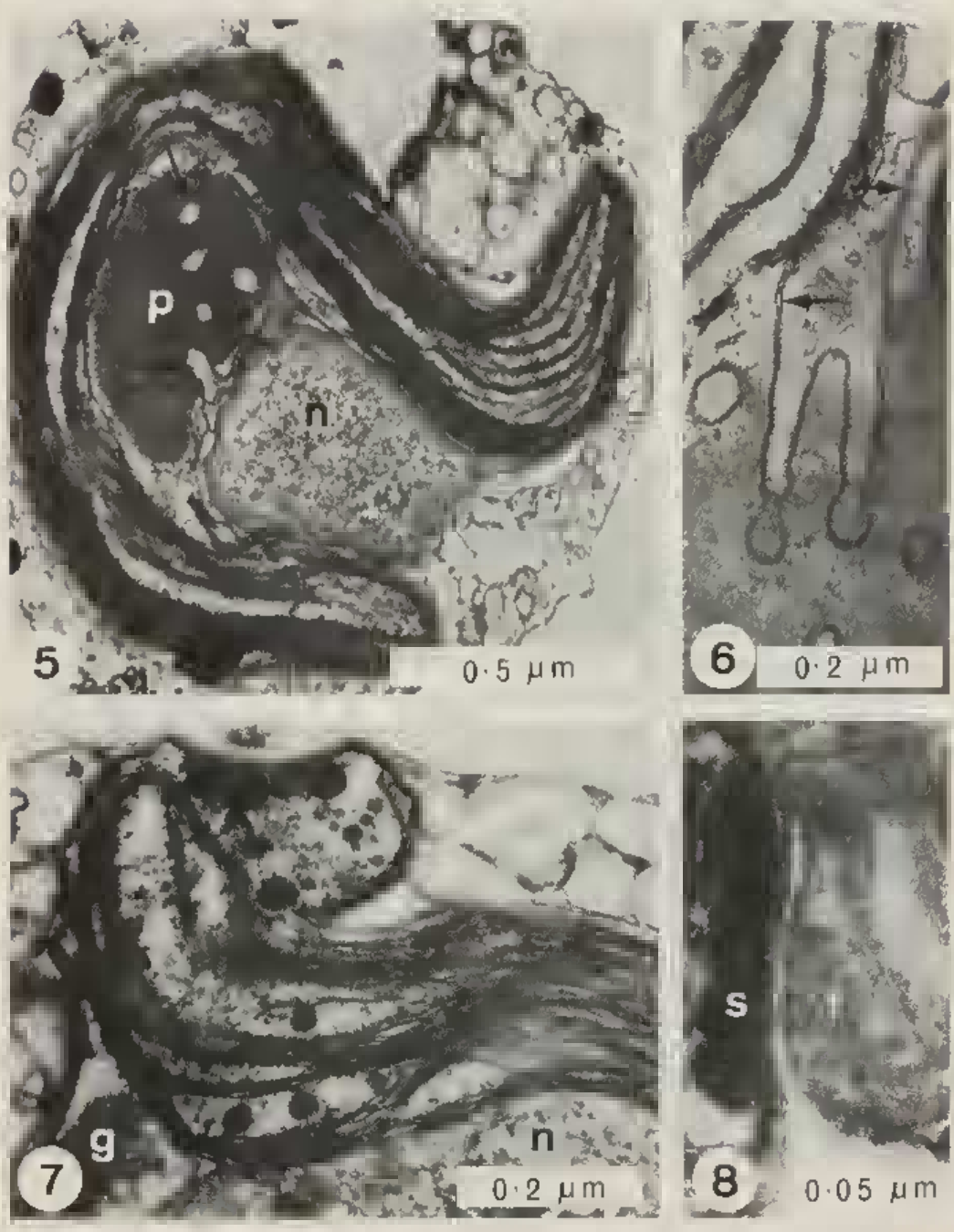

Plate II . electron micrographs 5-3. Fig. 5 · Thin section, circa transverse, showing plastid curved around nucleus (n), and pyrenoid (p) with profiles of galleries (arrow). — Fig. 6 : Detail of pyrenoid with two galleries cut longitudinally. Arrow indicates double plastid boundary membrane. — Fig. 7 : Stigma in a bulbous outgrowth of the plastid; one layer of pigment globules adjacent to plasmalemma, nucleus (n), Golgi (g). — Fig. 8 . Stigma (s) and flagellar swelling (f) separated by a narrow split.

The undulating long flagellum of attached cells effectuates a stream of water towards the anterior of the cell. Small objects carried by this stream can be caught for a moment by a sudden twitch of the flagellum that folds around them. When the object is released, it may immediately flow down along the long flagellum until it is arrested in front of the cell by the short flagellum, which then is directed forwards and makes rotating movements. The final ingestion is done by a collar that arises from the nearby cell surface, and that closes like a dome over the victim. After that, the pseudopodium is retracted and the food vacuole can be observed in the cell body.

Particle feeding is selective. In our cultures, bacteria are taken, whereas other objects may provoke a twitch and some folding of the flagellum, but simply flow away after that.

The plastid, with stigma and pyrenoid, and the vesicles in the surface layer of the cell are characteristic features. The single golden-brown plastid is bent around the nucleus. The external pyrenoid (Pl. I, fig. 1) is positioned against the nucleus, at the concave side of the plastid. It is not refringent as was described for *O. pinguis* Conrad by CONRAD (1930). The pyrenoid and the nucleus can be seen separately in Giemsa-stained cells when these are suitable orientated. The plastid posesses a stigma near the base of the short flagellum (Pl. I, fig. 3). At one side of resting cells, the plastid lies much closer to the cell surface than at the other side. Swimming cells do not show this asymmetry.

Small mucoid bodies occupy large areas of the cell surface (Pl. I, fig. 1). Thin mucoid strands radiates from some cells (Pl. I, fig. 2). A few larger vesicles may bulge out of the cell surface (Pl. I, fig. 3). These vesicles are difficult to see, and do not contain ejectile disks as were described for *O. tuberculata* Hibberd by HIBBERD (1970) and for *O. crenata* Klebs by KALINA (1964).

ELECTRON MICROSCOPY

Plate I, fig. 4 gives a survey of most cell constituents : the nucleus, dictyosome, chrysolaminarin vacuole, and two profiles of the plastid. These constituents, and also the rhizoplast not shown, conform in their details and mutual relationships to the known cytology of *Ochromonas*. They will not be described further here.

The stigma occupies a speciel outgrowth of the plastid, outside the girdle lamella (Pl. II, fig. 7). This outgrowth is situated closely to the surface of the cell, being separated from the swelling on the short flagellum by a narrow slit, shown in Pl. II, fig. 8. The stigma is composed of one layer of pigment globules which fit closely together. Some loose globules below this layer may be plastoglobuli, and do not belong to the stigma itself.

The pyrenoid contains several galleries (Pl. II, fig. 5 and 6), which sometimes are branched, and which always terminate in a slight widening. They are filled with cytoplasm, for the gallery walls are formed by the double plastid-boundary membrane (Pl. II, fig. 6).

The plastid is surrounded by a cisterna of the endoplasmic reticulum that is directly continuous with the nuclear envelope, and this cisterna contains bundles of straight microtubules, all features that are common in Chrysophycean algae. Besides microtubules, bacteria are found inside the endoplasmic reticulum of *Ochromonas monicis*. Plate III, fig. 9 shows one bacterium cut lengthwise, and another cut across. The bacterial cell walls, ribosomes, and DNA fibrils are clearly defined. The E.R. nature of the cavity by which these bacteria are confined is indicated by the ribosomes attached to its boundary membrane. The bacterial ribosomes are somewhat smaller than the algal ribosomes. Plate III, fig. 10 shows bacteria near the edge of the plastid, inside the plastid associated endoplasmic reticulum.

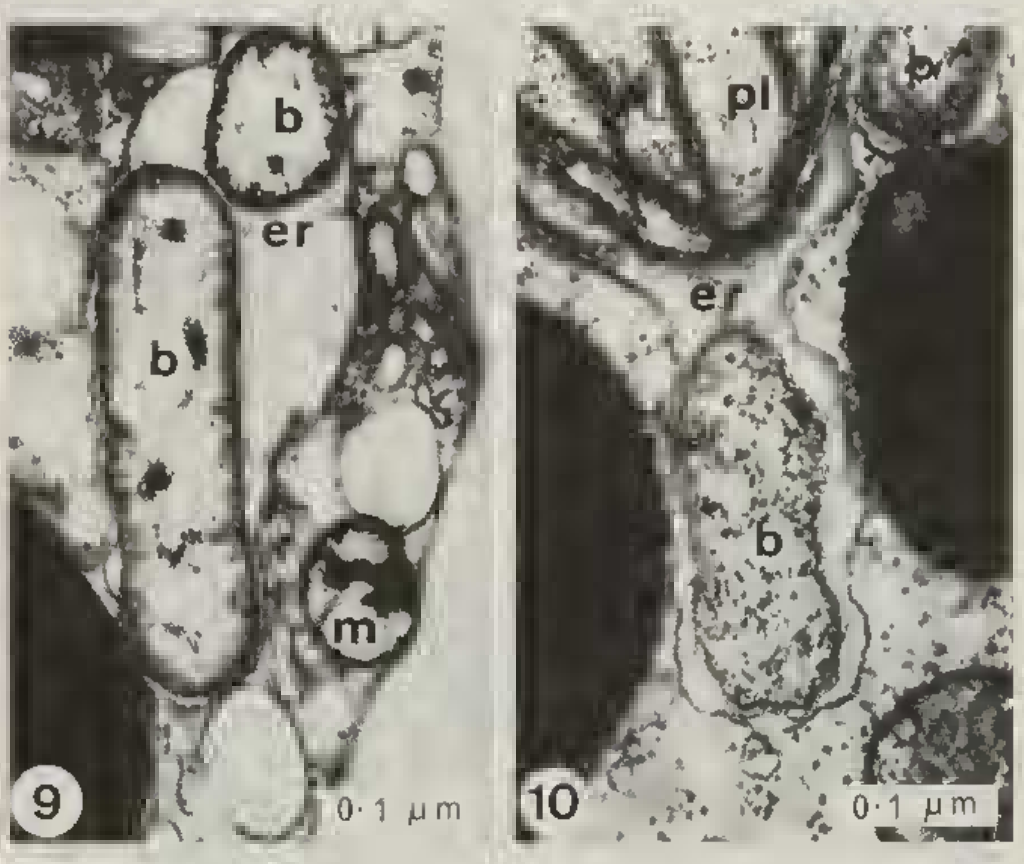

Plate III : electron micrographs 9 and 10. Fig. 9 : Two bacteria (b) inside a cisterna of endoplasmic reticulum (er); one cut longitudinally, the other across; mucoid body (m). — Fig. 10 : Bacteria (b) inside the plastid (p) associated endoplasmic reticulum (er).

We did not find any sign of attack by digestive enzymes in bacteria lying inside the endoplasmic reticulum. Apparently, these bacteria are endosymbionts. Preys digested to a varying extent are found in food vacuoles.

DISCUSSION

Five species of *Ochromonas* are reported to have pyrenoids : O. *pinguis* Conrad (CONRAD, 1930), O. *nannos* Skuja (SKUJA, 1939), O. *neustica* Skuja (SKUJA, 1948), O. *angulosa* Skuja (SKUJA, 1956), and O. *vischeri* Bourrelly (BOURRELLY, 1957). Only one of these species is reported to have a stigma : O. *angulosa*. However, the passage «*plerumque sine (raro cum?) pyrenoide et stigmate*» in the diagnosis of O. *angulosa* barely adds to the definition of the species. The other traits mentioned by SKUJA, and the cells portrayed by him, do not suggest any special affinity between O. *angulosa* and our material, let alone a specific identity.

The advantage, for the host, of harbouring endosymbionts is generally believed to consist in the excreted metabolites that can be used by the host. Cyanocobalamin could be such a useful product of endosymbiontic bacteria, for this substance is known to be required by some *Ochromonas* species, e. g. O. *malhamensis* Pringsheim (PRINGSHEIM, 1952) and O. *danica* Pringsheim (PRINGSHEIM, 1955), and it is synthesized by many marine bacteria (PROVASOLI & CARLUCCI, 1974). Cyanocobalamin-producing endosymbionts would make our particular *Ochromonas* strain less dependent on an external source of this substance.

The ecological role of *Ochromonas* species is complex. Their plastids make them primary producers. Like other algae, *Ochromonas* species may waste into tha water considerable amounts of glycollate, a result from the competition of oxygen with carbon dioxide for ribulose diphosphate in the photosynthetic pathway (TOLBERT, 1974). The reserve carbohydrate remains in solution, and leaks away at cell death. Hence, *Ochromonas* species probably contribute to the dissolved organic carbon in the seawater in two ways.

As a particle feeder, O. *monicis* has a position in the small food web, but this position is different from that of the protozoa (see WILLIAMS, 1981). The caught bacteria utilised organic substances dissolved in the seawater for their growth. and so bring some organic carbon excreted by the phytoplankton back into one of the phytoplankton components. It should be realized that not all organic carbon of the prey is equally important for photosynthesing predators. Almost certainly, the seizure of vitamins is not the main concern of algae that capture, and digest, bacteria. For, if sufficient vitamin-producing bacteria are present to prey upon, the amount of vitamin dissolved in the seawater will be sufficient to sustain the growth of the algae. The seizure of compounds of nitrogene or phosphorus seems to be a more beneficial result of the digestion of bacteria.

We suggest that O. *monicis* turns to phagocytosis when the nitrogen or phosphorus in the seawater becomes depleted.

DIAGNOSIS

Ochromonas monicis sp. nov.

Ochromonas chromatophora singulo, stigmate et pyrenoide. Tubi cytoplasma conti

nentes pyrenoidem penetrantes. Cellulae 5-9 µm longae, 4-7 µm latae, in statu natante oblongatae vel conoideae, in statu quieto plus minusve globosae. Unum flagellum 10-13 µm longum. Flagellum alterum 3 µm longum. Holotypus : Pl. I, fig. 1-3 et Pl. II, fig. 5-8.

Ochromonas with a single plastid, a stigma, and a pyrenoid. Tubes that contain cytoplasm penetrating the pyrenoid. Cells 5-9 µm long and 4-7 µm broad, in the swimming state oblong or cone shaped, in the resting state more or less round. One flagellum 10-13 µm long. The other flagellum 3 µm long. Holotype : Pl. I, fig. 1-3 and Pl. II, fig. 5-8.

ACKNOWLEDGEMENTS

We wish to thank Professor C. van den Hoek for reading the manuscript, M. W. Arkink for the micrograph of Pl. I, fig. 2, and Mr M. Veenhuis for technical advice on the electron microscopy. We enjoyed the research facilities of the Department of Ultrastructure Biology, at Haren.

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